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Digestion kinetics of low, intermediate and highly branched maltodextrins produced from gelatinized starches with various microbial glycogen branching enzymes

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ABSTRACT

Twenty-four branched maltodextrins were synthesized from eight starches using three thermostable microbial glycogen branching enzymes. The maltodextrins have a degree of branching (DB) ranging from 5 % to 13 %. This range of products allows us to explore the effect of DB on the digestibility, which was quantified under conditions that mimic the digestion process in the small intestine. The rate and extent of digestibility were analyzed using the logarithm of the slope method, revealing that the branched maltodextrins consist of a rapidly and slowly digestible fraction. The amount of slowly digestible maltodextrin increases with an increasing DB. Surprisingly, above 10 % branching the fraction of slowly digestible maltodextrin remains constant. Nevertheless, the rate of digestion of the slowly digestible fraction was found to decline with increasing DB and shorter average internal chain length. These observations increase the understanding of the structural factors important for the digestion rate of branched maltodextrins.

1. Introduction

Starch is the main carbohydrate energy source used by a wide variety of organisms, including humans. Many plants produce starch in the form of small granules, in which the two glucose polymers amylose and amylopectin are tightly packed together. Amylose is a virtually linear polymer of D-glucose units linked through α-1,4-glycosidic bonds with occasionally an α-1,6-glycosidic branch. Amylopectin is a branched polymer made of linear chains of D-glucose units linked *via* α-1,4-glycosidic bonds. The branches are formed by α-1,6-glycosidic linkages, with the degree of branching being approximately 3–5 % (Buleon, Colonna, Planchot, & Ball, 1998). Upon consumption, starch is initially digested in the mouth and esophagus by salivary α-amylase and subsequently in the small intestine by a combination of pancreatic α-amylase and brush border enzymes (Dhital, Warren, Butterworth, Ellis, & Gidley, 2017; Zhang & Hamaker, 2009).

Native starch granules have a slow digestible character as the densely packed amylose and amylopectin molecules form crystalline and amorphous regions thereby limiting the enzyme access, thus delaying enzymatic hydrolysis of the glycosidic linkages (Zhang, Ao, & Hamaker, 2008; Zhang, Ao, & Hamaker, 2006; Zhang, Venkatachalam, & Hamaker, 2006). However, starch is usually not consumed as intact granules, but as an ingredient in a food product that has seen a form of thermally processing, leading to the loss of the granular structure and allowing for rapid hydrolysis by digestive enzymes upon consumption (Butterworth & Ellis, 2019; Edwards & Warren, 2019; Svihus & Hervik, 2016).

Based on their degradation pattern starches are classified into three types: rapidly digestible starch (RDS, digested in the first 20 min), slowly digestible starch (SDS, digested between 20 and 120 min) and resistant starch (RS, not digested within 120 min) (Englyst, Englyst, Hudson, Cole, & Cummings, 1999). Starches with a high SDS content release their glucose at a lower rate over an extended period, thereby lowering the risk of developing type 2 diabetes and common chronic diet-related diseases (Kittisuban, Lee, Suphantharika, & Hamaker, 2014; Lee et al., 2008, 2016; Sim, Quezada-Calvillo, Sterchi, Nichois, & Rose, 2008).

To modulate the digestibility of gelatinized starch, the molecular structure of the amylose and amylopectin has to be changed in such a way that they are hydrolyzed at a lower rate (Li et al., 2016). One strategy is to change the branch density, *i.e.* increase the number of α-1,6-glycosidic bonds (Lee et al., 2013), as they are hydrolyzed slower than α-1,4-glycosidic bonds (French & Knapp, 1950; Kerr, Cleveland, & Katzbeck, 1951; Tsujisaka, Fukumoto, & Yamamoto, 1958). The branch

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density of starch can be increased by two types of enzymatic treatment: either using glycogen branching enzymes (GBE), which cleave α-1,4-glycosidic bonds and create new α-1,6 branches (Borovsky, Smith, & Whelan, 1976; Lee et al., 2008; Miao, Jiang, Jin, & BeMiller, 2018) or using β-amylases, which increase the ratio of α-1,6/α-1,4-glycosidic linkages by trimming the nonreducing ends of α-1,4-glucan chains till the first branch is reached (Kaplan & Guy, 2004; Kaplan, Sung, & Guy, 2006; Scheidig, Fröhlich, Schulze, Lloyd, & Kossmann, 2002). Corn starch modified by GBE and/or β-amylase was shown to be degraded at a lower rate than unmodified starch (Kittisuban et al., 2014; Lee et al., 2013).

In this report, eight native starches were each converted by three different microbial GBEs, resulting in twenty-four branched maltodextrins with varying degree of branching (DB), chain length distribution (CLD) and average internal chain length (AICL). This range of structural diverse branched maltodextrins allows us to explore the effect of the DB, CLD and AICL on the digestibility. *In-vitro* digestion revealed that the products are composed of a rapidly and slowly digestible maltodextrin fraction. Intriguingly, whereas the rate of digestion of the rapidly digestible maltodextrin is basically independent of the DB, the digestion rate of the slowly digestible maltodextrin fraction declines with an increasing DB and shorter AICL.

2. Materials and methods

2.1. Materials

Potato starch, waxy potato starch (Eliane C100), and tapioca starch were provided by Avebe (Groningen, Netherlands). Corn starch (Duryea, Maizena) was bought from a local supermarket. Pea starch was obtained from Roquette (Lestrem, France). Rice starch was purchased from Sigma-Aldrich (Zwijndrecht, Netherlands). Waxy corn starch was provided by Ingredion (Westchester, USA). Waxy rice starch was purchased from Beneo (Mannheim, Germany). Pancreatic α-amylase (EC 3.2.1.1, 16 U/mg solid) was obtained from Sigma-Aldrich (Zwijndrecht, Netherlands). Cluster Dextrin (4.2 % degree of branching; unpublished results) is manufactured by Ezaki Glico, and was purchased through the internet (Bulkpowders.nl). Isoamylase (EC 3.2.1.68, specific activity 260 U/mg), pullulanase M1 (EC 3.2.1.41, specific activity 34 U/mg), amyloglucosidase AMG from *Aspergillus niger* (EC 3.2.1.3, 3260 U/mL) and β-amylase (EC 3.2.1.2, specific activity 10,000 U/mL) were obtained from Megazyme (Wicklow, Ireland). The oligosaccharide kit was purchased from Sigma-Aldrich (Zwijndrecht, Netherlands). The genes coding for the GBEs of *Thermococcus kodakarensis* KOD1 (TkGBE), *Rhodothermus marinus* (RmGBE) and *Petrotoga mobilis* (PmGBE) were expressed in *E. coli* BL21 (DE3) and purified as reported (Zhang, Leemhuis, & van der Maarel, 2019).

2.2. Preparation of branched maltodextrins

Starches were gelatinized in 5 mM phosphate buffer pH 6.5 for TkGBE or pH 7.0 for RmGBE and PmGBE at a concentration of 0.125 % (w/v) and then heating under stirring. After boiling for 20 min the starch solutions were autoclaved at 121 °C for 20 min to completely gelatinize the starches. The hot starch solutions were directly transferred in a preheated water bath. When the temperature had decreased to the reaction temperatures, the GBEs were added. The incubations were performed at the enzyme's optimal reaction temperature, TkGBE at 70 °C and 30 μg/mL; RmGBE at 65 °C and 3 μg/mL; PmGBE at 50 °C 3 μg/mL. The activity of these three GBEs has been reported previously (Zhang et al., 2019). After 24 h reaction, the GBEs were inactivated by boiling for 20 min, and the modified starches were freeze dried for further analysis.

2.3. High performance anion exchange chromatography

Oligosaccharide analyses were carried out by high performance anion exchange chromatography (HPAEC) on a Dionex ICS-3000 system (Thermo Scientific, USA) equipped with a 4 × 250 mm CarboPac PA-1 column. A pulsed amperometric detector with a gold electrode and an Ag/AgCl pH reference electrode were used. The system was run with a gradient of 30 − 600 mM NaAc in 100 mM NaOH at 1 mL/min. Chromatograms were analyzed using Chromeleon 6.8 chromatography data system software (Thermo Scientific). A mixture of glucose, maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose, and maltoheptaose was used as reference for qualitative determination of elution time of each component. The detector response for DP2 to DP7 relative to glucose were calculated from the above reference.

2.4. Average chain length and average internal chain length analysis

The branched maltodextrin products were debranched by dissolving 2 mg of the products in 1 mL 5 mM sodium acetate buffer pH 5.0 supplemented with 5 mM $CaCl_2$. To 500 μL of this solution 0.7 U isoamylase and 0.5 U pullulanase were added and incubated at 40 °C for 16 h. The debranched samples were analyzed by HPAEC, revealing the chain length distribution (CLD). The average chain length (ACL) was calculated from the peak area of HPAEC profiles, after correction for signal response.

The chains of branched starch are classified into three types, A, B and C. The A-chains are linear unbranched chains attached to B-chains *via* their C_1; B-chain are further branched by another chain; and C-chains carry a reducing end. Each branched molecule has a single reducing end. The AICL of B-chains was determined by treating the samples with the exo-acting enzyme β-amylase, followed by debranching. β-amylase trims the external α-glucan chains from the nonreducing end leaving either one or two glucosyl residues from the branch for the B-chains. Thus, the average overhang beyond the outmost branch after β-amylase treatment is 1.5 glucosyl residues for B-chains. The A-chains are trimmed down to either a maltose or maltotriose chain (Rashid et al., 2016).

The branched α-glucans (2 mg/mL) were treated with β-amylase (5 U/mL) at 40 °C in 50 mM phosphate buffer (pH 6.5) for 24 h. Following β-amylase inactivation by boiling for 10 min, the pH was set to 4.0–5.0 with diluted HCl and the material was debranched with 0.7 U/mL isoamylase and 0.5 U/mL pullulanase at 40 °C for 16 h. Following inactivation of the debranching enzymes by boiling for 10 min, the samples were analyzed by HPAEC. The chain length distribution was compared to the chain length distribution without β-amylase treatment. The percentage of A-chains was calculated as the ratio between two times the peak area of maltotriose (A-chains were hydrolyzed to maltose and maltotriose by β-amylase) and the total peak area. The AICL was calculated as follow:

$$\text{AICL} = \frac{\text{BAM} - 1.5}{1/(1 - \text{A\%})} - 1$$

BAM: the average chain length of β-amylase treated α-glucans. A%: the percentage of A-chains in α-glucans.

2.5. H^1-NMR spectroscopy

^{1}H-NMR spectra were recorded at a probe temperature of 323 K on a Varian Inova 500 spectrometer (NMR Center, University of Groningen). Before analysis, samples were exchanged twice in D_2O (99.9 atom% D, Sigma-Aldrich Chemical) with intermediate lyophilization, and then dissolved in 0.6 mL D_2O. Spectra were processed using MestReNova 14.0 software (Mestrelabs Research SL, Santiago de Compostella, Spain), using a Whittaker Smoother baseline correction and zero filling to 32 k complex points. The DB was calculated by dividing the area of

the α-1,6-glycosidic linkage signal by the combined areas of the α-1,4- and α-1,6-glycosidic linkage signals.

2.6. In-vitro digestion

The digestibility of the products was evaluated by incubating them with a mixture of pancreatic α-amylase and amyloglucosidase. Pancreatic α-amylase powder was dissolved (2.55 mg/mL) in 100 mM citrate buffer (pH 6.0) with 10 mM $CaCl_2$ and subsequently 0.95 μL/mL amyloglucosidase was added into the α-amylase solution. Undissolved material was removed by centrifugation at 10,000x*g* for 10 min. The products (25 mg/mL) were dissolved in ultrapure water. The carbohydrate content of each sample was quantified by the Anthrone method (Dreywood, 1946). Digestion was performed with 1632 U α-amylase and 124 U amyloglucosidase per gram of starch at 37 °C. The enzyme unit is referred to the product instruction from the company. The rate of digestion was followed by taking aliquots of 10 μL into 390 μL pure water in time, and directly stopping further digestion by boiling for 5 min. The amount of glucose formed was quantified by the GOPOD method (Vasanthan, 2001). The slopes of digestibility curves were calculated at each time point throughout the incubation, converted to logarithmic form and then fitted to the first-order kinetic model (Butterworth, Warren, Grassby, Patel, & Ellis, 2012; Sorndech et al., 2015). Accurate estimate of the pseudo rate constant (k) and the total digestible starch ($C\infty$) are obtainable from plots of LOS against time.

3. Results and discussion

3.1. Synthesis and structure of branched maltodextrins

Eight regular and waxy gelatinized starches were modified with three different thermostable microbial GBEs, yielding twenty-four branched maltodextrins with a DB ranging from 5 to 13 % (Table 1). The TkGBE generated branched maltodextrins with a relative low DB (4.9–6.2 %), whereas the Rm/PmGBE produced highly branched maltodextrins (DB of 10–13 %). The chain length distributions (CLDs) of all eight branched maltodextrins made by TkGBE are very similar, having a bimodal profile with maxima at DP 7 and DP 12, and no side chains longer than DP 16. PmGBE conversion, in contrast, results in unimodal CLDs, with a maximum at DP 6 (tapioca, rice and waxy potato starch) or at DP 7 (pea, corn, potato, rice and waxy corn starch). The CLDs of the RmGBE products are somewhere in between with a maximum at DP 7 and 9, except for the waxy potato and rice starch derived products which have a maximum at DP 6 and 8 (Fig. 1 & Supplementary information Figs. S1–S3). Interestingly, the CLDs reveal that the Rm/PmGBE products, with the higher DB, have a small fraction of longer chains, which are absent in the TkGBE products (Fig. 1 & Supplementary information Fig. S1–S3). Overall it may be concluded that the CLD profile of the branched maltodextrins is predominantly determined by the GBE used, while the type of starch substrate makes only a small contribution. The Rm/PmGBE products have shorter side chains than the TkGBE products, and the fraction of short chains (DP < 13) increases with increasing DB (Tables 1 and 2). Importantly, the branched maltodextrins with the lower DB have around 16 % A-chains, while the highly branched maltodextrins have a much higher fraction of A-chains, on average 32 and 37 % for RmGBE and PmGBE, respectively (Table 1). Based on the structural model of glycogen proposed by Meléndez et al., 1997, it is assumed that the A-chains are situated on the outside of the branched maltodextrins and that the A-chains have a DP higher than the AICL. Taking into consideration that the Rm/PmGBE branched maltodextrins have significantly less medium length chains (DP 13 − 24) than the TkGBE products (Table 2), it is proposed that these highly branched maltodextrins have more but considerably shorter A-chains than the TkGBE branched maltodextrins. The structural analysis clearly shows that the type of GBE, and not the type of starch, is the dominating factor in determining the structural properties of the branched maltodextrins.

Table 1
Structural properties of the branched maltodextrins derived from eight starches modified with *T. kodakarensis*, *R. marinus*, and *P. mobilis* GBEs. Degree of branching (DB), average chain length (ACL), average internal chain length (AICL) and percentage of A-chains. The analyses were performed in triplicate.

	T. kodakarensis GBE			
Substrate	DB (%)	ACL (DP)	AICL (DP)	A-chain (%)
Pea	6.2 ± 0.6	9.7 ± 0.6	5.2 ± 0.2	16.8 ± 1.9
Corn	5.2 ± 0.3	9.9 ± 0.4	5.3 ± 0.3	16.5 ± 1.8
Potato	5.3 ± 1.2	10.0 ± 0.8	5.3 ± 0.5	16.3 ± 3.7
Rice	5.1 ± 0.7	9.7 ± 0.9	5.4 ± 0.4	16.3 ± 2.4
Tapioca	5.8 ± 0.7	9.8 ± 0.9	5.5 ± 0.3	16.1 ± 3.3
Waxy potato	5.2 ± 0.7	10.0 ± 0.5	5.5 ± 0.4	15.3 ± 3.3
Waxy rice	4.9 ± 0.4	10.0 ± 0.7	5.5 ± 0.2	16.4 ± 2.8
Waxy corn	5.0 ± 0.2	10.0 ± 0.8	5.4 ± 0.2	15.9 ± 3.2
Average ± SD	5.3 ± 0.4	9.9 ± 0.1	5.4 ± 0.1	16.2 ± 0.5
	R. marinus GBE			
Substrate	DB (%)	ACL (DP)	AICL (DP)	A-chain (%)
Pea	10.9 ± 0.2	9.4 ± 0.1	3.7 ± 0.3	37.3 ± 1.2
Corn	10.3 ± 0.1	9.6 ± 0.1	3.9 ± 0.2	37.8 ± 0.8
Potato	10.1 ± 0.2	9.2 ± 0.1	3.9 ± 0.2	37.6 ± 1.1
Rice	10.4 ± 0.2	9.7 ± 0.1	4.0 ± 0.1	36.9 ± 1.5
Tapioca	10.5 ± 0.0	9.7 ± 0.2	3.9 ± 0.2	38.5 ± 2.0
Waxy potato	10.1 ± 0.3	9.8 ± 0.1	4.2 ± 0.3	36.6 ± 1.3
Waxy rice	10.5 ± 0.5	9.9 ± 0.1	4.2 ± 0.1	35.7 ± 1.6
Waxy corn	10.2 ± 0.1	9.9 ± 0.1	4.0 ± 0.3	36.9 ± 0.9
Average ± SD	10.4 ± 0.3	9.7 ± 0.2	4.0 ± 0.2	37.2 ± 0.8
	P. mobilis GBE			
Substrate	DB (%)	ACL (DP)	AICL (DP)	A-chain (%)
Pea	13.1 ± 0.3	8.5 ± 0.2	2.9 ± 0.1	32.8 ± 1.6
Corn	12.9 ± 0.5	8.7 ± 0.4	2.7 ± 0.2	35.2 ± 3.3
Potato	12.6 ± 0.5	8.7 ± 0.2	3.0 ± 0.1	32.1 ± 1.9
Rice	13.0 ± 0.8	8.8 ± 0.1	2.9 ± 0.1	32.2 ± 1.3
Tapioca	12.8 ± 0.6	8.8 ± 0.2	3.1 ± 0.1	32.1 ± 1.5
Waxy potato	12.8 ± 0.7	8.8 ± 0.4	3.3 ± 0.2	30.8 ± 2.1
Waxy rice	12.5 ± 0.4	8.8 ± 0.1	3.4 ± 0.2	30.2 ± 1.9
Waxy corn	12.7 ± 0.5	8.9 ± 0.1	3.3 ± 0.4	30.2 ± 2.5
Average ± SD	12.8 ± 0.2	8.8 ± 0.1	3.1 ± 0.2	32.0 ± 1.6

GBEs act on long linear chains, cleaving α-1,4-glycosidic bonds and transferring the cleaved off chain to either the same or a different amylose or amylopectin molecule resulting in a new α-1,6 branches. The DB of the branched maltodextrins produced with a GBE is inversely correlated to the ACL and AICL (Bertoft, Laohaphatanalert, Piyachomkwan, & Sriroth, 2010; Laohaphatanaleart, Piyachomkwan, Sriroth, & Bertoft, 2010; O'Sullivan & Perez, 1999). This trend is also observed for the eight branched maltodextrins made by each of the three different GBEs, though the correlation is rather weak (Fig. 2C–H), because each of the GBEs creates products with a narrow range of DBs. However, the three different GBEs used in this study synthesize branched maltodextrins with a wide range of DBs (Table 1). Importantly, this variation in DBs enables a better exploration of the effect the DB has on the structural and digestion properties. Comparison of all 24 branched maltodextrins together, with low, intermediate and highly branched products, reveals a clear inverse linear correlation between the DB and AICL (R^2 of 0.98) (Fig. 2A). The DB and ACL are also inversely correlated (Fig. 2B), although the correlation is weaker (R^2 = 0.66), which is in line with the observation made by Li et al. (2018). Although it cannot be excluded that the effect is only driven by the type of GBE used, we think that also branched maltodextrins made by other GBEs, with different DBs, will fit the trend lines shown in Fig. 2A/B. Overall it can be concluded that the higher the DB is, the shorter the AICL is of branched maltodextrins.

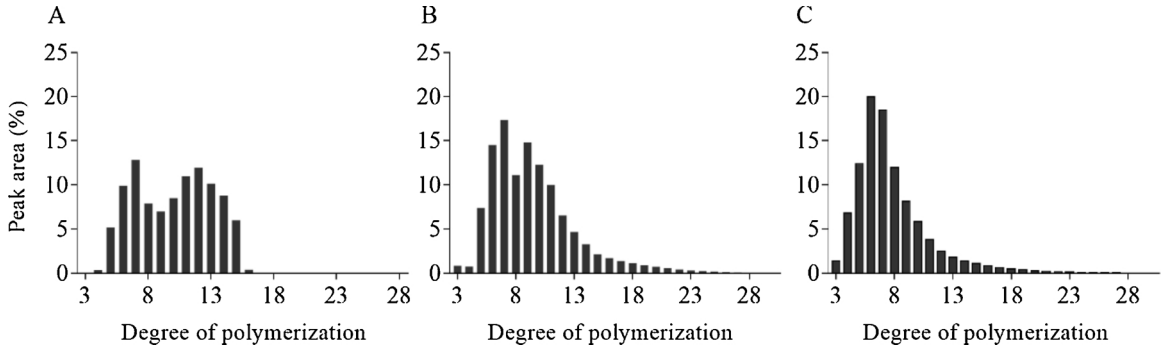

Fig. 1. Chain length distribution (CLD) of the branched maltodextrins derived from gelatinized tapioca starch by the GBE action of *T. kodakarensis* (A), *R. marinus* (B) and *P. mobilis* (C). The CLD profiles of the branched maltodextrins derived from the other seven starches are shown in supplementary information (Figs. S1–S3).

Table 2
CLD of the branched maltodextrins produced from the eight gelatinized starches with three different GBEs.

	T. kodakarensis GBE		
Substrate	DP < 13 (%)	DP13 – 24 (%)	DP > 25 (%)
Pea	78.7 ± 0.1	21.3 ± 0.1	0
Corn	80.2 ± 0.1	19.8 ± 0.1	0
Potato	74.8 ± 0.3	25.2 ± 0.3	0
Rice	75.8 ± 0.2	24.1 ± 0.2	0
Tapioca	74.8 ± 0.1	25.2 ± 0.1	0
Waxy potato	76.5 ± 0.1	23.5 ± 0.1	0
Waxy rice	75.8 ± 0.2	24.2 ± 0.2	0
Waxy corn	73.9 ± 0.2	26.1 ± 0.2	0
Average ± SD	76.3 ± 2.1	23.7 ± 2.1	0
	R. marinus GBE		
Substrate	DP < 13 (%)	DP13 – 24 (%)	DP > 25 (%)
Pea	88.6 ± 0.1	11.1 ± 0.1	0.4 ± 0.1
Corn	87.2 ± 0.1	12.4 ± 0.1	0.4 ± 0.1
Potato	89.7 ± 0.2	10.9 ± 0.2	0.5 ± 0.2
Rice	86.2 ± 0.2	13.4 ± 0.1	0.4 ± 0.1
Tapioca	86.4 ± 0.1	13.1 ± 0.1	0.5 ± 0.1
Waxy potato	85.5 ± 0.1	13.9 ± 0.1	0.6 ± 0.1
Waxy rice	84.5 ± 0.1	15.0 ± 0.2	0.5 ± 0.1
Waxy corn	84.6 ± 0.1	14.9 ± 0.1	0.5 ± 0.1
Average ± SD	86.6 ± 1.8	13.1 ± 1.6	0.5 ± 0.1
	P. mobilis GBE		
Substrate	DP < 13 (%)	DP13 – 24 (%)	DP > 25 (%)
Pea	94.9 ± 0.1	5.1 ± 0.1	0
Corn	94.4 ± 0.1	5.6 ± 0.1	0
Potato	93.3 ± 0.1	6.7 ± 0.1	0
Rice	92.5 ± 0.1	7.3 ± 0.1	0.2 ± 0.1
Tapioca	92.6 ± 0.1	7.3 ± 0.1	0.1 ± 0.1
Waxy potato	92.2 ± 0.1	7.7 ± 0.1	0.1 ± 0.1
Waxy rice	93.6 ± 0.2	6.3 ± 0.3	0
Waxy corn	92.3 ± 0.1	7.7 ± 0.1	0
Average ± SD	93 ± 1.0	6.7 ± 1.0	0.1 ± 0.1

3.2. In-vitro digestion

The rate of digestion of the 24 different branched maltodextrins produced in this study was explored, using Cluster Dextrin and granular potato starch as controls. Cluster Dextrin is a mildly branched (4.2 % DB) α-glucan derived from waxy corn starch and is faster digested than the branched maltodextrins made in this study in the *in-vitro* digestion test (Fig. 3). The TkGBE branched maltodextrins, having a DB of 4.9–6 %, released the highest amount of glucose in 360 min (74–89 %), and showed most variation in the rate of digestion, possibly due to the relatively large variation in the DB of these products (Table 1). The more highly branched Rm/PmGBE products, composed of shorter chains, released substantially less glucose (60–70 %) than the TkGBE products (Fig. 3).

The rate of glucose release was fitted according to the logarithm of slope (LOS) plot approach used by Butterworth et al. (2012) and Sorndech et al. (2015) for the analysis of starch hydrolysis. The LOS plots of the digestion data from the 24 branched maltodextrins and Cluster Dextrin revealed an initial fast digestion phase, followed by a phase of slow digestion (Fig. 4). The LOS plots provide two kinetic constants k_1 and k_2, indicating the susceptibility of the branched maltodextrins to the action of pancreatic α-amylase and amyloglucosidase in the fast and slow phase, respectively. Surprisingly, the rate constant k_1 of the initial phase of rapid digestion was rather similar for all the branched maltodextrins (Fig. 5A, Table S1), even though these products have very different DB.

Excitingly, it was observed that the rate of digestion of the slow phase (k_2) is strongly declining with an increasing DB and decreasing AICL. The average k_2 values decreased nearly threefold from 2.9×10^{-3} min^{-1} for TkGBE, to 1.72×10^{-3} min^{-1} for RmGBE) and 1.04×10^{-3} min^{-1} for PmGBE (Fig. 5B). Thus, initially the A-chains and the exterior parts of the B-chains are trimmed down resulting in a rapid release of glucose, the fast phase represented by k_1. Then, a branch has to be removed, which occurs relatively slowly, before a short internal α-1,4-chain becomes available, which is then quickly trimmed till the next branch is reached. This cycle is then repeated. Thus, with longer AICL more glucoses are released between two branches, resulting in a higher k_2, compared to branched maltodextrins with a shorter AICL. Because the AICL is inversely correlated with the DB, the more branched maltodextrins are slower digested.

From the discontinuity in the LOS plot also the fraction of rapidly and slowly digestible maltodextrin (RDM and SDM, respectively) can be calculated, as described by Patel, Day, Butterworth and Ellis (2014). The amount of RDM and SDM was calculated from eight products of each GBE. The eight branched maltodextrins made by TkGBE consist of on average 38 % RDM and 62 % SDM (Fig. 6). As expected, the average fraction of SDM is significantly higher for the more branched maltodextrins synthesized by RmGBE and PmGBE, being around 64.5 %. However, to our surprise the RmGBE and PmGBE products have an identical fraction of SDM, despite the fact that the PmGBE products are clearly more branched (10 % vs 13 %). This observation indicates that the amount of slow digestible maltodextrin synthesized by GBEs initially increases with an increasing DB, and reaches a plateau at a branched density of about 10 %.

4. Conclusion

Once consumed starches and branched maltodextrins are degraded by the combined action of α-amylase and the brush boarder enzymes of the small intestine. It is assumed that the rate of digestion is declining with an increasing DB as α-1,6-glycosidic bonds are hydrolyzed at a

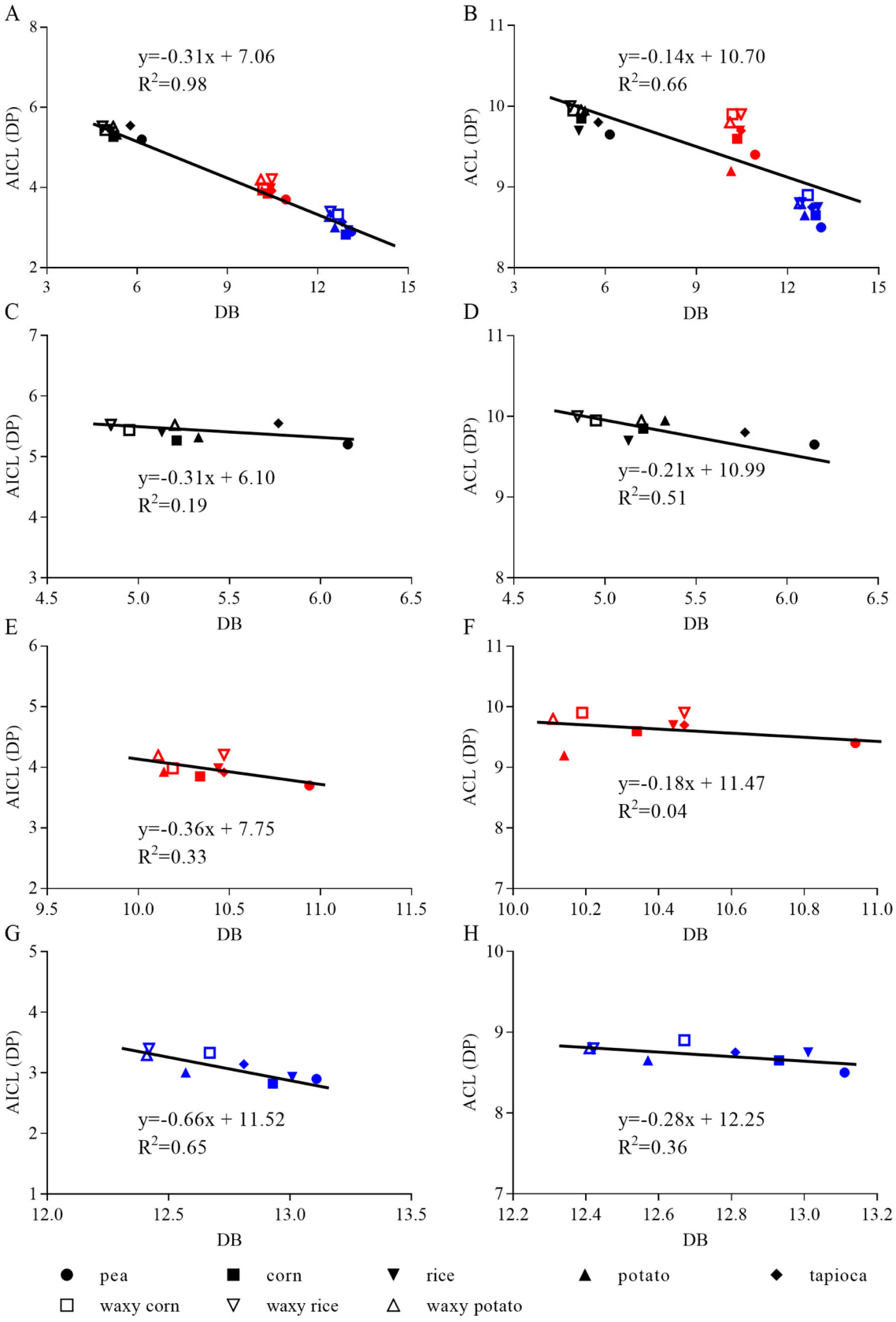

Fig. 2. Correlation among the DB, the AICL (A) and the ACL (B) of the 24 products made by the different GBEs, TkGBE (black), RmGBE (red) and PmGBE (blue). Panels C&D (TkGBE), E&F (RmGBE) and G&H (PmGBE) show the correlations for the individual GBEs (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

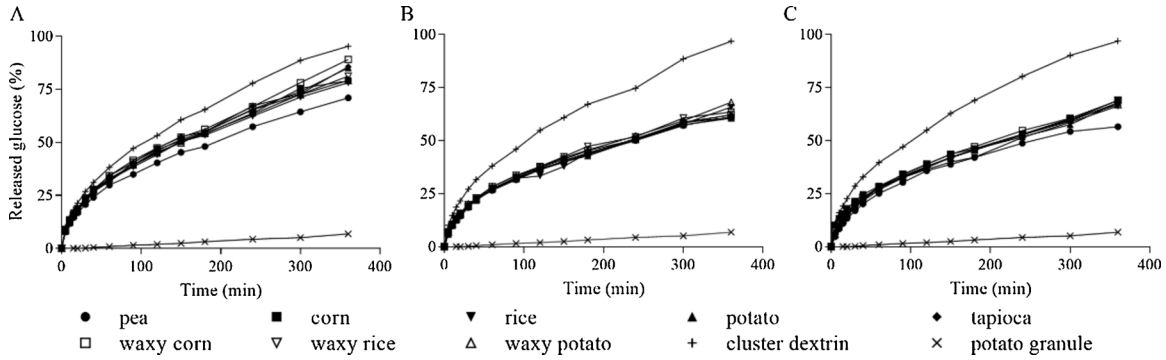

Fig. 3. *In-vitro* digestibility curves of the GBE modified starches. TkGBE (A), RmGBE (B) and PmGBE (C). Controls: Cluster Dextrin and potato starch granules.

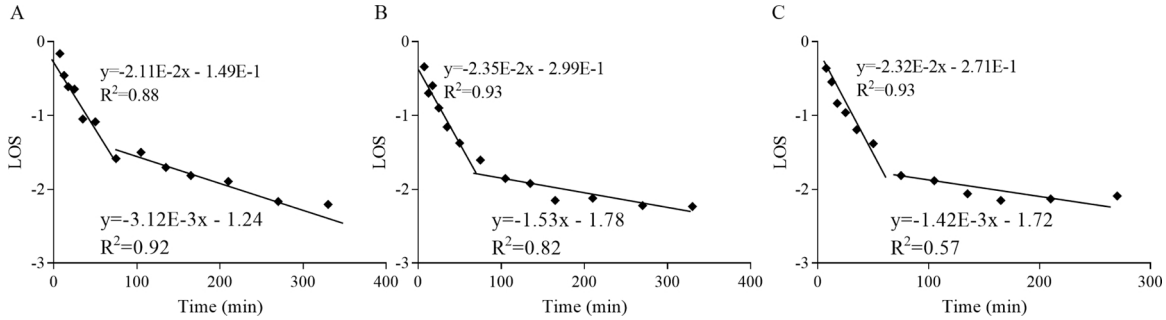

Fig. 4. LOS plot of the branched maltodextrins generated from tapioca starch by TkGBE (A), RmGBE (B) and PmGBE (C). LOS plots of all other branched maltodextrins are shown in the supplementary information (Figs. S4–S6).

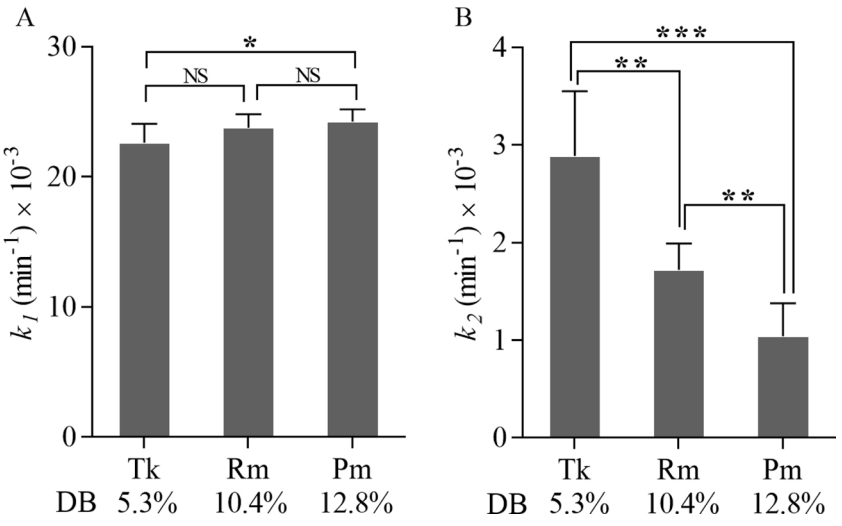

Fig. 5. The average k_1 (A) and k_2 (B) values of the branched maltodextrins made by TkGBE, RmGBE and PmGBE. The average degree of branching (DB) is shown below the bars. The three average k_1 values are not significantly different from each other, whereas all three k_2 are significantly different from each other. NS (not significant): $P > 0.05$; *: $0.01 < P < 0.05$; **: $0.001 < P < 0.01$; ***: $P < 0.001$. The unpaired *t*-test was applied. A correlation table is provided in the supplementary information (Table S1).

lower rate than α-1,4-glycosidic bonds. Our analysis of 24 branched maltodextrins reveals that the digestion occurs rapidly in the initial fast phase followed by a phase of slow digestion. The branched maltodextrin products thus consist of a rapidly and slowly digestible fraction. To our surprise the fraction of slowly digestible maltodextrin did not raise once the DB exceeds 10 %, the upper limit being 65 % of slowly digestible maltodextrin.

Intriguingly, our results show that the digestion rate of the rapidly digestible maltodextrin is virtually independent of the DB. Contrary, there is strong and negative correlation between the DB and the digestion rate of the slowly digestible maltodextrin. Thus, even though the yield of slowly digestible maltodextrin did not further increase above 10 % branching, an even higher DB considerably lowered the digestion rate of the slowly digestible maltodextrin. It is thus highly relevant to synthesize α-glucans with the highest possible DB and shortest possible AICL, because such α-glucans are expected to be more slowly converted into glucose in our gastrointestinal tract which is positive from a health perspective.

CRediT authorship contribution statement

Xuewen Zhang: Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing. **Hans Leemhuis:** Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing. **Marc J.E.C. van der Maarel:** Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

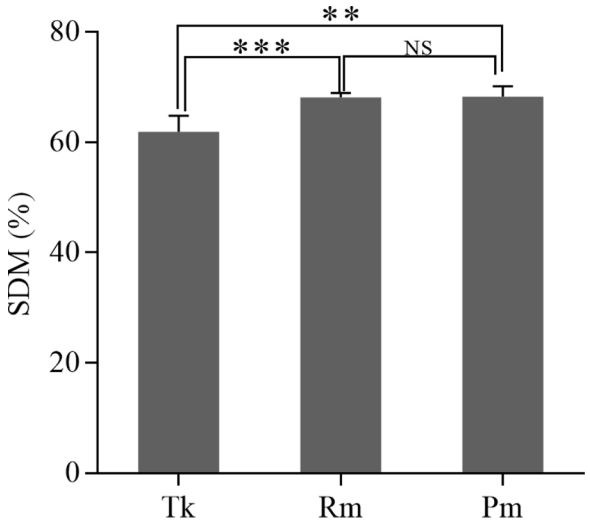

Fig. 6. The average amount of slowly digestible maltodextrim (SDM) of all eight branched maltodextrins made by each of the GBEs. The amount of SDM is significantly different between Rm/PmGBE and TkGBE. Note that by definition the sum of the percentage of SDM and rapidly digestible maltodextrin (RDM) is 100 %. NS (not significant): $P > 0.05$; **: $0.001 < P < 0.01$; ***: $P < 0.001$. The unpaired *t*-test was applied.

Declaration of Competing Interest

The authors declare that they don't have any conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.carbpol.2020.116729.

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